

Role of Fluctuations in a Snug-fit Mechanism of KcsA Channel Selectivity

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A tetrameric constellation of four TTVGYG amino acid sequences comprises the selectivity filter of the KcsA channel shown in Fig. 1. This signature sequence is strongly conserved across a variety of K^+ channels [1]. In each binding site, e.g., the S_2 site defined by $(VG)_4$, the ion is coordinated by four carbonyl oxygens from above and four from below. One mechanism for selectivity based on the atomic structure of the KcsA channel invokes the size difference between K^+ and Na^+ , and the molecular complementarity of the selectivity filter with the larger K^+ ion [1]. An alternative view holds that size-based selectivity is precluded because atomic structural fluctuations are greater than the size difference between these two ions [2]. This view is based on extensive free energy calculations that show local interactions leading to structural flexibility of the binding site provides selectivity for K^+ . Turning off the electrostatic repulsions between the carbonyl oxygens, while retaining ion-carbonyl oxygen electrostatic attractions shifts the selectivity in favor of Na^+ .

We note that these two views are not mutually exclusive. For example, artificially turning off electrostatic repulsions between the carbonyl oxygens while maintaining the ion-carbonyl oxygen attractions collapses the filter around either ion, and the extent of this collapse will depend on the size of the ion. The combination of size differences and fluctuations is also seen in the bulk hydration properties of these two ions. Notably, the partial molar volume of K^+ in water is positive, while the partial molar volume of Na^+ in water is negative [3].

Here we follow [2] in studying a simplified model of the S_2 site of selectivity filter to examine the role of fluctuations in KcsA channel selectivity. The equilibrium selectivity of the filter can be characterized by the difference in the interaction free energy for transferring Na^+ from water into the selectivity filter compared to K^+ . Thus, we study

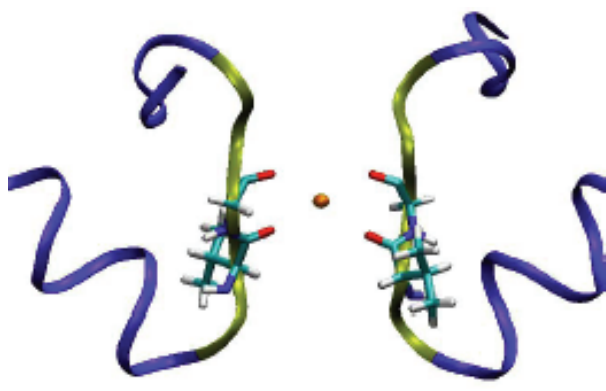
$$\begin{aligned}\Delta\mu^{ex} &= [\mu_{Na^+}^{ex}(filter) - \mu_{K^+}^{ex}(filter)] \\ &\quad - [\mu_{Na^+}^{ex}(aq) - \mu_{K^+}^{ex}(aq)] \\ &= \Delta\mu^{ex}(filter) - \Delta\mu^{ex}(aq),\end{aligned}\quad (1)$$

where $\mu_X^{ex}(aq)$ ($X = K^+, Na^+$) is the hydration free energy of the ion, and $\mu_X^{ex}(filter)$ is the analogous quantity in the selectivity filter. The excess chemical potential, μ_X^{ex} , is that part of the chemical potential that would vanish if intermolecular interactions were to be neglected. μ_X^{ex} to the ideal gas result at the same density and temperature. A standard state could be adopted in which $\Delta\mu^{ex}$ would vanish, but that would not change any physical consideration.

The potential distribution theorem [4, 5] tells us how μ_X^{ex} may be calculated from the probability density distribution of the binding energy, ϵ , of the X ion to the medium. This function is generated with the ion and the medium fully coupled at temperature $T = 1/k_B\beta$ where k_B is Boltzmann's constant. In the present approach, ion positions contributing to the sample are those corresponding to its natural motion in water or in the ion-protein system. If the probability density function is described by a Gaussian of mean $\langle\epsilon\rangle$ and variance $\sigma^2 = \langle(\epsilon - \langle\epsilon\rangle)^2\rangle$, then

$$\mu_X^{ex} = \langle\epsilon\rangle + \beta\sigma^2/2. \quad (2)$$

Note that in sampling the fully-coupled system the term $\beta\sigma^2/2$ raises the chemical potential above the mean binding energy $\langle\epsilon\rangle$. As a check of Eq. 2, we also computed $\Delta\mu^{ex}$ by transforming K^+ to Na^+ on the basis of a coupling-parameter integration through 20 intermediate states. Details of the theory, calculations, and molecular dynamics simulations are given in the supplemental material.



The bulk hydration free energies set the baseline for filter selectivity as is evident in Eq. 1. Coupling-parameter integration yields -20.7 kcal/mol for $\Delta\mu^{ex}(aq)$, in good agreement with [6]. A gaussian model gives -23.0 kcal/mol. It is estimated that $\Delta\mu^{ex}$ is 6 kcal/mol [2], or roughly three times smaller than the difference in bulk hydration free energies. Thus the hydration thermodynamic properties in bulk solution provide the biggest contribution to Na^+/K^+ selectivity.

Figure 2 shows the distribution of ion-carbonyl oxygen binding energies. It is clear that the mean binding energy of Na^+ with the filter is much lower than that for K^+ . The distribution of energies for Na^+ is also much broader. The magnitude of the implied difference in fluctuation contributions is comparable to the net selectivity, and this difference is the final contribution discriminating Na^+ from K^+ . Analysis of those fluctuation contributions shows that filter configurations conducive to binding Na^+ are constricted and rare occurrences in the ensemble of conformations favorable to binding K^+ . We conclude that energetically favorable ion binding in the selectivity filter occurs for both Na^+ and K^+ . For Na^+ , this is achieved at the expense of enhanced energetic and positional fluctuations compared to K^+ , the ion considered to have a better geometrical fit in the selectivity filter. In this sense, the difference in size between Na^+ and K^+ , and the molecular complementarity of the selectivity filter with K^+ play

an important role in channel selectivity. Fluctuations induced by the more favorable binding of the smaller Na^+ in the selectivity filter must also be considered, however, and it is this contribution to the binding free energy that discriminates K^+ from Na^+ . The key result, that has not been appreciated so far [1, 2], is the *joint* association of strong binding, constriction of the filter, and enhanced

fluctuations in the case of the smaller Na^+ ion. The constriction of the filter by the Na^+ ion, relative to the natural fit of K^+ ion, is consistent with the experimental fact that the partial molar volume of $\text{K}^+(aq)$ is positive, while the partial molar volume of $\text{Na}^+(aq)$ is negative.

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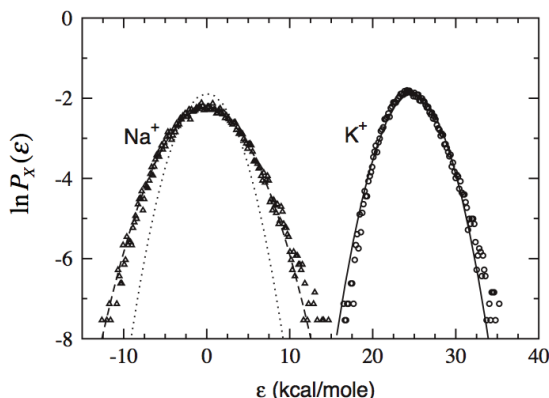


Fig. 1.
Depiction of the channel filter. Only two of the four chains are shown.

Fig. 2.
Normalized distributions of ion-carbonyl oxygen binding energies, for $X = \text{Na}^+$ or K^+ . The abscissa is the binding energy differences from the mean value for the Na^+ case. Solid curves are gaussian fits to the data. The dashed curve is the K^+ gaussian model translated leftward to superpose the K^+ gaussian model on the Na^+ data, showing that that the Na^+ distribution is significantly wider.